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Physiological Characteristics and Cold Resistance of Five Woody Plants in Treeline Ecotone of Sygera Mountains

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Abstract: Investigating the distribution of internal physiological indicators and the cold resistance of woody plants in the alpine treeline ecotone is of great ecological importance to explain the mechanism of alpine treeline formation. Less research has been conducted on the cold resistance mechanisms of alpine treeline woody plants than on commercial crops. In this paper, five different tree species in the alpine treeline ecotone of the Sygera Mountains were used as the research objects and the leaves, branches, and roots of 19 woody plants were collected in the non-growing season (November) of 2019. Their non-structural carbohydrate content (soluble sugar and starch), malondialdehyde, hydrogen peroxide (H_2O_2), proline, superoxide dismutase, and peroxidase levels were measured. The contents of C, N, P, and K elements were analyzed, along with the distribution characteristics of physiological indices and organs of various woody plants and their relationship to plant nutrients. Results showed that the MDA (5.46 ± 1.95 μg·g^{-1}) and H_2O_2 (4.11 ± 0.76 mmol·g^{-1}) of tree root organs and the MDA (3.03 ± 2.05 μg·g^{-1}) and H_2O_2 (4.25 ± 1.03 mmol·g^{-1}) of shrub leaf organs were higher than those of other organs, indicating that under the stress of low temperatures, the root organ of arbor species and the leaf organ of shrub species experienced the most damage. Osmotic substances, particularly soluble sugars, play a crucial role in the response of the woody plants in Sygera Mountains to low-temperature stress. Plant nutrients could enhance plant stress resistance by further activating the activity of the antioxidant system and increasing the synthesis of osmotic substances. This study hypothesized that the stress on the root organs of the arbor species in the treeline ecotone may not be repaired in time, which may be a key mechanism for the formation of the alpine treeline in the Sygera Mountains.

Keywords: Sygera Mountains; treeline ecotone; cold resistance; plant nutrients; root organ

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1. Introduction

The alpine treeline, as the top limit of forest distribution, is an ecological transition zone between the alpine canopy tree forest and the shrub meadow [1]. Environmental elements, including temperature, humidity, solar radiation, and atmospheric pressure, drastically changes with the increase in altitude. Questions of why woody plants fail to thrive or grow badly at a particular altitude? and how the currently existing woody plants will react to environmental changes? have been raised [2–4]. The majority of literature is predicated on the link between climate and treeline. Low-temperature stress, for example, has a significant effect on plant growth and low soil temperature and heat deprivation could limit tree growth and prevent its spread to higher altitudes [5–7]. Li discovered that extreme low temperatures in Southeastern Tibet could inhibit the growth of xylem in trees [8]. In addition to temperature, additional environmental elements [9], such as water stress [10], wind and light [11], and solar radiation [12], have been shown to influence the growth of trees according to related studies. Non-climatic factors, such as nutrient usage [13], biological interactions [14], and land-use patterns [15], influence alpine treeline species, in addition to meteorological constraints. After plateau plants are subjected to environmental stress, organs such as leaves, branches, and roots could clearly reflect the effect of low temperature on plant growth and development [16]. Meanwhile, their physiologically active substances, such as antioxidants and antioxidant enzyme activities, are closely related to plant stress resistance [17]. In general, soluble sugar, malondialdehyde (MDA), proline (Pro) and protective enzymes (superoxide dismutase [SOD], peroxide dismutase [POD] and catalase [CAT]) are the key indicators for measuring cold resistance in plants [18,19]. MDA is one of the products of membrane lipid peroxidation and its concentration indicates the degree of cell membrane lipid peroxidation and the resiliency to stress [19]. H_2O_2 is an essential component of reactive oxygen species (ROS) and its concentration is positively linked with plant cell damage [20]. As osmotic regulators, Pro and soluble sugar could boost plants' resistance to environmental stress [19]. Various studies have concluded that the concentration of soluble sugar in plants is proportional to their ability to resist freezing [21,22]. The activity of antioxidant enzymes and the increase in antioxidant content (such as SOD, POD, and CAT) are crucial for cold resistance in plants [23,24].

Few studies were conducted on the physiological characteristics of woody plants, particularly in the alpine treeline ecotone, despite the fact that current research on plant cold tolerance focuses mostly on agriculture and forestry crops [25,26]. In the Sygera Mountains in southeastern Tibet, *Abies georgei* var. *smithii* and *Sabina saltuaria* dominate the treeline arbor layer, whereas *Rhododendron aganniphum*, *Rhododendron nyingchiense,* and *Rhododendron nivale* dominate the shrub layer. The upper limit of the distribution of *Abies georgei* var. *smithii* did not change significantly against the backdrop of global warming, which could be offset by other factors. The alpine treeline of Sygera Mountains consists of two species, *Abies georgei* var. *smithii* and *S. saltuaria*, making it one of the world's highest treelines [27]. The distribution of internal physiological indicators and the capacity for cold resistance could verify and partially explain the mechanism of alpine treeline establishment. In the present study, the levels of soluble sugar, starch, Pro, H_2O_2, and MDA in the leaves, branches, and roots of five distinct woody plants in the treeline ecotone of Sygera Mountains were investigated. With the aim to explain the formation of alpine treelines in the Sygera Mountains from the perspective of plant physiology and ecology and the mechanism of distribution limitation, studies on the distribution characteristics of physiological indicators, such as SOD and POD activities and their cold resistance, lay the groundwork for research on the growth adaptation strategies of the physiological characteristics of major forest tree species. Similarly, the present study postulated that the stress on the root organs of arbor tree species in the treeline ecotone could not be restored in time, which may be a key process in the creation of the alpine treeline in the Sygera Mountains.

2. Materials and Methods

2.1. Study Area

Sygera Mountains (93°12′–95°35′ E, 29°10′–30°15′ N) are situated in the southeast of the Qinghai-Tibet Plateau, Bayi District, Nyingchi City, Tibet Autonomous Region, at the confluence of the Nienqing Tanggula Mountains and the Himalayas, with an elevation of around 5200 m. In Sygera Mountains, the alpine treeline ecotone mostly refers to the belt between 4300 and 4600 m in altitude. The annual mean temperature in this location is −1.25–1.80 °C. The average monthly temperature in the warmest month is 8.71–9.32 °C and the average monthly temperature in the coldest month ranges from −7.23 °C to −9.06 °C. Up to 999–1056 mm of precipitation is primarily concentrated during the growth season. In the alpine and cold zones, the predominant forest vegetation type is the mixed zone of arbor-shrub-meadow and the dominating tree species are *Abies georgei* var. *smithii* and *S. saltuaria*.

R. aganniphum, *R. nyingchiense*, and *R. nivale* are the most prevalent tree species in the shrub layer. The prominent herbaceous plant species include *Cassiope fastigiata*, *Bergenia purpurascens*, and *Rhodiola fastigiata*. The type of soil is subalpine (shrub) meadow soil.

2.2. Sample Collection

In late November of 2019, a 30 m × 30 m quadrature was established in the typical ecological distribution area of *Abies georgei* var. *smithii* and *S. saltuaria* in the treeline ecotone of Sygera Mountains and the diameter at breast height and tree height of all target plants within the quadrature were measured (Table 1). Five arbor layer plants with average growth vigor (*Abies georgei* var. *smithii* and *S. saltuaria*) were chosen and samples of their leaves, branches, and roots were taken. Among the shrub layer plants, *R. aganniphum*, *R. nyingchiense*, and *R. nivale* predominated. Three healthy, robustly developing plants with comparable ground diameter, height, and crown breadth were chosen and samples of their leaves, branches, and roots were gathered.

Table 1. Main woody plants and their basic information in the alpine treeline ecotone of Sygera Mountains.

Tree Species	Function Type	Altitude/m	Slope/°	CD/%	TH/m	DBH/cm	CW(E-W)/m	CW(N-S)/m
Abies georgei var. *smithii* (AG)	E-N-A	4300	25	80	13.2 ± 0.6	134.3 ± 4.3	3.4 ± 0.2	3.6 ± 0.5
Sabina saltuaria (SS)	E-N-A	4420	25	50	10.2 ± 0.7	90.8 ± 4.5	3.2 ± 0.1	3.2 ± 0.2
Rhododendron aganniphum (RA)	E-B-S	4300	-	-	-	-	-	-
Rhododendron nyingchiense (RNY)	E-B-S	4420	-	-	-	-	-	-
Rhododendron nivale (RNI)	E-B-S	4420	-	-	-	-	-	-

Note: CD, canopy density; TH, tree height; DBH, diameter at breast height; CW (E–W), crown width (east–west); CW (N–S), crown width (north–south); E-N-A, evergreen-needleleaf-arbor; E-B-S, evergreen-broadleaf-shrub. "-" represents missing data.

Sampling of leaves and branches: Branches and leaves from the east, west, north, and south directions in the middle of the canopy were taken and leaves (1–3 years old) and branches (1–2 years old) were collected to represent the entire canopy and were then combined to create samples of individual leaves and branches. Root sampling: Using the excavation method, fine roots (diameter of 2 mm) were chosen as the root samples after washing the fine roots recovered from the soil strata with a depth of 5–30 cm. The samples were then placed in an incubator with an ice box for preservation and delivered back to the laboratory in time for processing. The collected samples were subjected to pretreatments, such as dividing and cleaning, before being rapidly fixed in an oven at 105 °C for 15 min, dried at 80 °C to constant weight, crushed and passed through a 1 mm sieve, packaged in Ziplock bags, and stored in a desiccant box for testing.

2.3. Biochemical Parameters

In the leaves, branches, and roots of five woody plants, the sample determination primarily analyzes MDA, H_2O_2, Pro, POD, SOD, soluble sugar, and starch. MDA concentration was determined using the thiobarbituric acid colorimetric method [28]; pro concentration was determined using the ninhydrin colorimetric method [29]; starch and soluble sugar concentrations were determined using the anthrone sulfate method [16]; H_2O_2 concentration was determined using the built-in biological kit method [30]; and the activities of POD (EC 1.11.1.7) and SOD (EC1.15.1.1) were determined in accordance with the method of Giannopolitis and Ries (1977) [31]. Elemental analyzer method (Vario EL cube CHNOS Elemental Analyzer, Elementar Analysensysteme GmbH, Hanau, Germany) was used to determine the C and N contents in various plant organs and HNO_3-H_2O_2

digestion-ICP-OES method (iCAP 6300 ICP-OES Spectrometer, Thermo Fisher, USA) was used to determine the P and K contents in various plant organs [16].

2.4. Data Analysis

The interaction between different tree species and organs was examined using nested multivariate ANOVA and multiple comparisons were conducted using Duncan's test. The data were statistically analyzed using SPSS 23.0, with the significance level set to 0.05 for all the results passing the tests for variance homogeneity and normal distribution. The relationship between plant physiology and nutrient indicators was examined using Pearson's correlation analysis and Origin Pro Lab 2021 (OriginLab, version 2021, MA, USA) was employed for figuring. The membership function was used to standardize the trait comprehensive index score value of each variety on each principal component:

$$\mathrm{U}\left(X_j\right)=\frac{\left(X_j-x_{min}\right)}{\left(x_{max}-x_{min}\right)}\ \mathrm{j}=1,2,3\ldots n \tag{1}$$

In the formula, X_j represents the score value of the $\mathrm{J_{th}}$ factor, X_{min} represents the minimum score value of the $\mathrm{J_{th}}$ factor, and X_{max} represents the maximum score value of the $\mathrm{J_{th}}$ factor.

$$W_j=\frac{P_i}{\sum_{j=1}^{n} P_i}\ \mathrm{j}=1,2,3\ldots n \tag{2}$$

In the formula, W_j represents the importance degree of the $\mathrm{J_{th}}$ common factor in all common factors and P_i represents the contribution rate of the $\mathrm{J_{th}}$ common factor in each variety.

$$D_i=\sum_{j=1}^{n}\left\{U(X_i)\times W_j\right\}\ \mathrm{i}=1,2,3\ldots k \tag{3}$$

In the formula, D_i is the comprehensive evaluation value of the cold resistance of the species evaluated by comprehensive indicators under low temperature conditions and k is the number of species.

3. Results

3.1. Analysis of the Variation Source for Each Physiological Index between Tree Species and Organs

The effect of tree species, organs, and the interplay between tree species and organs on each physiological index varied (Table 2). MDA was significantly influenced by the relationship between tree species and organs (Type IIISS = 73.668) but not by organs ($p > 0.05$). H_2O_2 was affected by tree species, organ, and the interaction between tree species and organ, with organ being the most affected (Type IIISS = 83.228). POD was significantly influenced by tree species (Type IIISS = 391,266.440), organs, and the interaction between tree species and organs ($p < 0.05$). SOD was significantly influenced by the interaction between tree species and organs (Type IIISS = 283,956.021), tree species (Type IIISS = 189,144.989), but not by organs ($p > 0.05$). The organ had the greatest effect on soluble sugar, starch, and soluble sugar/starch, although soluble sugar was unaffected by the tree species and organ interaction and NSCT were unaffected by the tree species.

Table 2. Source of variation analysis for each physiological index.

Parameter	Sources of Variation	Type IIISS	DF	MS	F	*p*
MDA	Tree species (T)	43.177	4	10.794	19.317	0.000
	Organ (O)	2.343	2	1.172	2.097	0.136
	T×O	73.668	8	9.208	16.479	0.000
H_2O_2	T	30.199	4	7.550	24.381	0.000
	O	83.228	2	41.614	134.391	0.000
	T×O	62.436	8	7.804	25.204	0.000
Pro	T	168,159.362	4	42,039.840	19.249	0.000
	O	290,921.055	2	145,460.527	66.604	0.000
	T×O	391,266.440	8	48,908.305	22.394	0.000
POD	T	1,672,339.493	4	418,084.873	145.484	0.000
	O	25,492.132	2	12,746.066	4.435	0.018
	T×O	105,025.950	8	13,128.244	4.568	0.000
SOD	T	189,144.989	4	47,286.247	10.785	0.000
	O	17862.737	2	8931.368	2.037	0.143
	T×O	28,3956.021	8	35,494.503	8.095	0.000
SS	T	1741.269	4	435.317	8.719	0.000
	O	2540.121	2	1270.061	25.437	0.000
	T×O	416.552	8	52.069	1.043	0.420
ST	T	1848.201	4	462.050	5.796	0.001
	O	18,258.872	2	9129.436	114.513	0.000
	T×O	9252.173	8	1156.522	14.507	0.000
SS/ST	T	16.735	4	4.184	5.881	0.001
	O	55.921	2	27.961	39.304	0.000
	T×O	27.485	8	3.436	4.830	0.000
NSCT	T	902.101	4	225.525	1.807	0.145
	O	7309.347	2	3654.674	29.288	0.000
	T×O	9188.871	8	1148.609	9.205	0.000

Note: Type IIISS is the sum of squares of deviations and the greater the value, the stronger is the factor's influence.

3.2. Physiological Index Distribution Characteristics of Five Dominant Woody Plants in the Treeline Ecotone

Significant differences were found between tree species and organs in terms of physiological indicators. When comparing the same organ with different tree species (Figure 1), the concentrations of MDA, H_2O_2, and Pro in shrub leaf organs were generally higher than those in arbor species. In contrast, the POD and SOD contents of shrub leaf organs were lower than those of tree leaf organs. The concentrations of MDA, H_2O_2, POD, and NSC in the root organs of arbor species were greater than those of arbor species. Comparison of the MDA, H_2O_2, and Pro contents of different organs of the same tree species (Figure 1) showed that the MDA, H_2O_2, and Pro contents of root organs in arbor species were greater than those of the other organs, whereas in shrub species, these contents of leaf organs were greater. The contents of SOD and POD in root organs were greater than those in leaf and branch organs. In all tree species, leaf organs had a higher SS/ST ratio than other organs.

3.3. Analysis of the Cold Resistance of Five Dominant Woody Plants in the Treeline Ecotone

SPSS data processing software was used to standardize each individual index and the standardized data and index coefficient of each principal component comprehensive index obtained through principal component analysis were multiplied to determine the comprehensive index score [CI(x)] for each tree species. In accordance with the formula, the membership function value (x) of the two principal component comprehensive indexes of each material and then the weight of each principal component comprehensive index were calculated. After the weights of the two principal component comprehensive indices were obtained, which were 0.722 and 0.278, the formula was used to calculate the comprehensive score value (D) of each material, which is the cold resistance capability of the material. The cold resilience of every tree species was ranked by its D value. The greater the D value was, the greater a tree species' cold resistance. By categorizing the D values of each tree, Table 3 shows that the leaf cold resistance of different tree species is as follows: *R. nivale* (0.916) > *R. nyingchiense* (0.877) > *R. aganniphum* (0.545) > *S. saltuaria* (0.278) > *Abies georgei* var. *smithii* (0.183). Meanwhile, the cold resilience of the roots of several tree species is depicted in Table 4 as follows: *Abies georgei* var. *smithii* > *R. nyingchiense* > *R. nivale* > *S. saltuaria* > *R. aganniphum*.

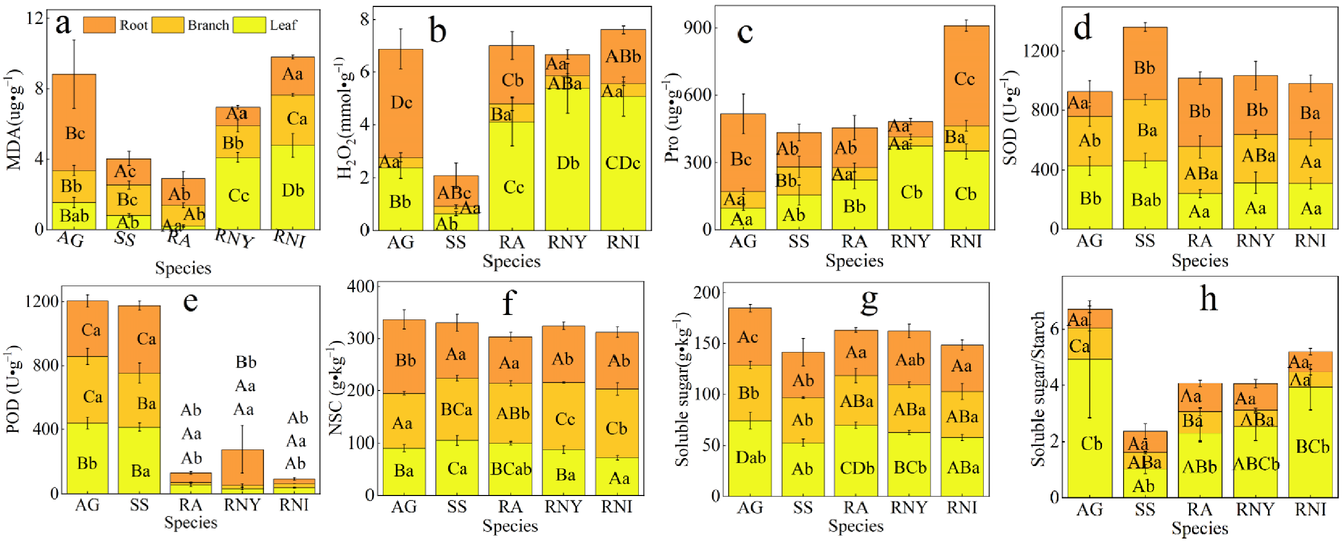

Figure 1. Distribution characteristics of physiological indexes of five dominant woody plants in different organs and tree species in the treeline ecotone of Sygera Mountains. (**a**–**h**) represents the content of MDA, H2O2, Pro, SOD, POD, NSC, soluble sugar and soluble sugar/starch in different organs and tree species, respectively. RA stands for *Rhododendron aganniphum*, RNY stands for *Rhododendron nyingchiense* and RNI stands for *Rhododendron nivale*. AG stands for Abies georgei var. smithii. SS stands for *Sabina saltuaria*. Different capital letters denote substantial variations between organs from the same tree species from different tree species, while different lowercase letters denote significant differences between organs from the same tree species from different organs.

Table 3. Common factor score C(x), membership function value U(x), and comprehensive evaluation value D of five tree species (leaf organs).

Parameter	C1	C2	U1	U2	D	Comprehensive Evaluation
Rhododendron nivale	1.98	0.59	1	0.7	0.916	1
Rhododendron nyingchiense	1.81	0.45	0.964	0.65	0.877	2
Rhododendron aganniphum	0.45	−0.96	0.674	0.21	0.545	3
Sabina saltuaria	−2.72	1.55	0	1	0.278	4
Abies georgei var. *smithii*	−1.53	−1.62	0.253	0	0.183	5
Weights	-	-	0.722	0.28	-	-

Note: "-" represents missing data.

Table 4. Common factor score C(x), membership function value U(x), and comprehensive evaluation value D of five tree species (root organs).

Parameter	C1	C2	U1	U2	D	Comprehensive Evaluation
Rhododendron nivale	3.67	0.32	1	0.713	0.928	1
Rhododendron nyingchiense	−1.01	1.14	0.095	1	0.323	2
Rhododendron aganniphum	−1.17	1.05	0.064	0.969	0.292	3
Sabina saltuaria	0	−1.72	0.29	0	0.217	4
Abies georgei var. *smithii*	−1.5	−0.79	0	0.325	0.082	5
Weights	-	-	0.748	0.252	-	-

Note: "-" represents missing data.

3.4. Analysis of the Correlation between Nutrients and Physiological Indicators of Five Major Woody Plants in the Treeline Ecotone

A correlation was observed between the nutrients and physiological indicators of the five woody plants in the crisscross zone at the treeline. As indicated in Figure 2, C was considerably favorably correlated with H_2O_2, Pro, SS, and SS/ST ($p < 0.05$) and strongly negatively correlated with POD, ST, and NSCT ($p < 0.05$); N, P, K, and S were significantly negatively correlated with POD, ST, and NSCT ($p < 0.05$). The stoichiometric ratio had no significant correlation with MDA, H_2O_2, Pro, or SOD ($p > 0.05$); C, N, P, and K were significantly positively correlated with SS ($p < 0.05$) and significantly negatively correlated with ST ($p < 0.05$). In addition, C:P, C:K, N:P, and N:K all showed a significant negative correlation with POD ($p < 0.05$), whereas C:N, C:P, and C:K were negatively correlated with SS/ST ($p < 0.05$).

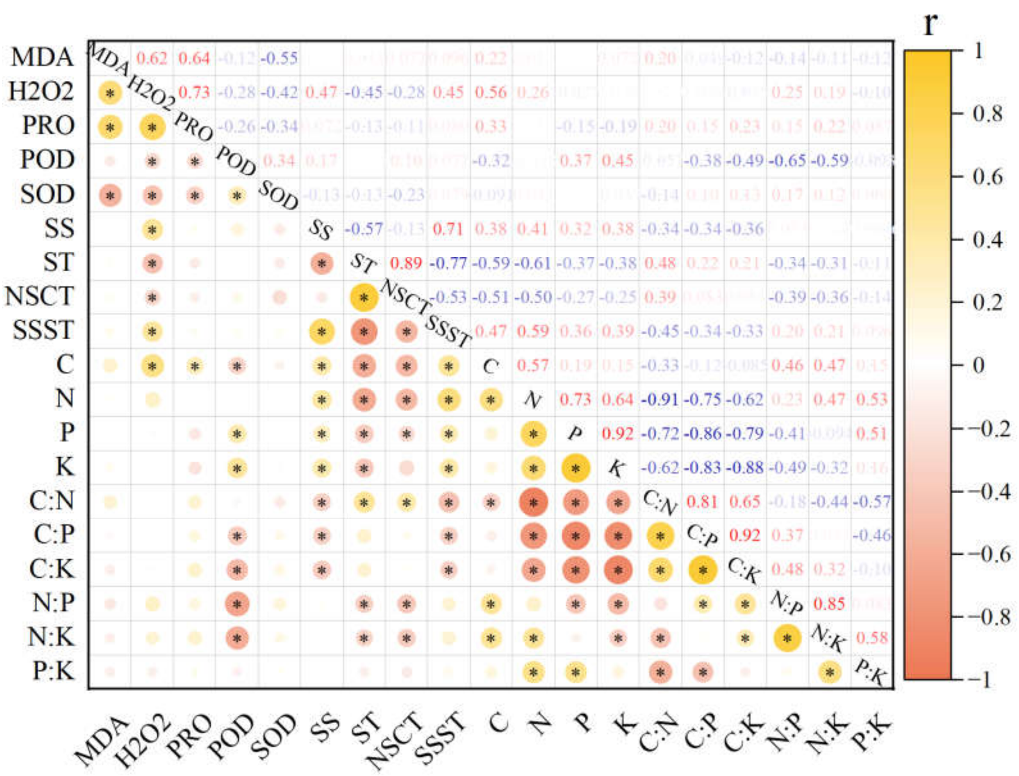

Figure 2. Correlation analysis of nutrients and physiological indicators of five dominant woody plants in the treeline ecotone of Sygera Mountains. SS stands for soluble sugar, ST stands for starch, and NSCT stands for the sum of soluble sugar and starch. * represents $p < 0.05$.

4. Discussion

4.1. Membrane Metabolites and Cold Resistance of Plants

The formation of ROS (such as H_2O_2) in plant organs as a result of stresses, such as drought or low temperature, results in membrane lipid peroxidation, which, in turn, results in the accumulation of MDA in the organs. Meanwhile, the content of MDA and H_2O_2 could be utilized to indicate the extent of damage to plant organ cells [20,32]. The greater the content of MDA and H_2O_2 is, the greater the extent of damage to plant cells [33]. It also reflects the physiological state of plant membrane lipids under cold stress [34]. During the non-growing season, the root organ of tree species are more damaged and the leaf organ of shrub species are more damaged and larger than other organs. This phenomenon may be due to the morphological characteristics of plants. All trees are evergreen needle-leaved species, whereas all shrubs are evergreen broad-leaved species. The leaf area of tree species is clearly lower than that of shrub species, resulting in less damage to tree species under low-temperature stress. This finding also suggests that the cold resistance of tree leaf organs is greater than that of shrub leaf organs, consistent with the conclusion of Cui [35], who revealed that the cold resistance of plant leaves is directly related to its morphological structure. This finding has also been confirmed by a study on the cold resistance of the membership functions of leaf organs of five tree species (Table 2). Although the cold resistance of the root organs of *Abies georgei* var. *smithii* was higher than that of *S. saltuaria* and other shrubs, the root organs of *Abies georgei* var. *smithii* experienced a more severe "cold foot effect" during the non-growing season [8,16], which increased the degree of cell damage.

4.2. Plant Cold Resistance and the Antioxidant System

Plants have evolved an antioxidant system to eliminate ROS and defensive enzymes to increase their cold tolerance in response to the accumulation of ROS in plant organs. SOD and POD are first-line antioxidants, which can quickly remove O_2- and limit the production of OH [36]. According to this study, the utilization of antioxidant enzymes varies between tree and shrub species. High levels of SOD and POD in each organ of arbor species showed that SOD and POD are mostly used in conjunction to ROS elimination in organs and high levels of SOD and low levels of POD in shrub species showed that SOD is primarily used to eliminate ROS in organs. According to the research of Liang [24] on North Chinese larch, plant cold tolerance is positively connected with increased enzyme activity in plants [37] and the POD concentration in leaves, branches, and root organs is higher than that of *S. saltuaria* and *Abies georgei* var. *smithii*. Manas Ranjan Sahoo et al. believed that SOD activity of plants under stress increased by 52.6% compared with that of control plants [32]. The POD content of the organs of different tree species did not significantly differ from one another (except *R. aganniphum*). The SOD content of *S. saltuaria* roots and leaves during the dormant season was the highest and that of *Abies georgei* var. *smithii* roots was the lowest, demonstrating that *S. saltuaria* had a superior environmental situation. The root system of *Abies georgei* var. *smithii* experiences severe low-temperature stress during the off-growing season, thus affecting its adaptability. Regardless of the leaves, branches, or roots, the POD activity of tree species was higher than that of shrub species. This finding showed that in the treeline ecotone, the external environmental stress suffered by trees is more severe than that by shrub species, necessitating an increase in the body's enzyme activity to overcome this disadvantage [23]. And this is also consistent with Perez's conclusion that under stress, plants possess rapid and effective antioxidant reaction ability, which indicates that they have stronger resistance to stress [38].

4.3. Plant Cold Resistance and Osmotic Adjustment Agents

Pro and soluble sugars operate as osmotic regulators and build up following plant stress, which could successfully maintain the stability of the cell membrane while lowering the water potential within the cell. Plants could also play a significant role in resisting difficult circumstances, such as low temperatures and drought stress, when they have sufficient NSC, Pro content, and SS/ST ratio [26,39,40]. According to Kavi et al., overproducing proline could increase plant's resilience to osmotic stress [41]. They also discovered that non-structural carbohydrates or soluble sugars are more abundant in plants with stronger cold tolerances. Accumulation could improve plant's resistance to cold [42]. In the present study, the distribution of NSC content among different tree species and organs was also well balanced and the Pro content of the roots and leaves of woody plants in the treeline crisscross zone was at a considerably high level. SS and ST were higher than those of other organs, consistent with Charrier's work [43], suggesting that woody plants in the treeline engraving increased their stress resistance by selecting to store more soluble sugars in leaf organs or converting starch into soluble sugars. By contrast, according to earlier findings, the stress on the root organs of tree species is greater than that on the leaf organs of shrub species. Shrub species may experience less stress if treeline species use a strategy that involves storing more soluble sugar in their leaf organs or turning starch into soluble sugar. No more permeable material could relieve the high-intensity stress on the root organs of tree species during the non-growing season [44] and such mechanism may affect the treeline and shrub species in the treeline ecotone, an essential determinant in the distribution.

4.4. Plant Nutrition and Frost Resistance

Plants employ various techniques to withstand environmental stress. According to similar studies, plant nutrients are directly associated with plant's cold tolerance [45] and a high C content could enhance plant's photosynthetic efficiency and adaptability to environmental changes [46]. N and P are essential components of organic substances in plants, including chlorophyll, protein, and nucleic acid. They are also the basis of genetic material and they play a crucial role in various physiological and metabolic activities, including plant growth, development, and differentiation [47,48]. K is an activator of numerous plant enzymes [49]. In the present study, the P and K contents of plants enhanced the synthesis of POD and the C, N, P, and K contents encouraged the synthesis of SS. Therefore, plant nutrients may enhance osmotic pressure in plant organs. This finding is congruent with the findings of Chen for 12 kinds of woody plants in various vertical climate zones of Sygera Mountains [16].

5. Conclusions

Same as the hypothesis, in the alpine treeline ecotone of Sygera Mountains, the root organs of tree species experience the most stress and damage and the leaf organs of shrub species experience the most stress and damage. It increases its own cold resistance by activating the antioxidant system's vitality and increasing osmotic substances, which are also positively regulated by plant nutrients. In time, the high SS/ST ratio of leaf organs could ease and restore the intense stress on the leaf organs of shrub species. Although arbor species also maintain a high level of SS/ST in leaf organs, they disregard the protection of root organs, making it impossible to alleviate and repair the high-intensity stress suffered by root organs. This finding also explains why shrub species are distributed at a higher altitude than tree species and provides theoretical support for shrub expansion under global climate change. Similarly, it recommends that the selection of shrub species should be prioritized when greening high-altitude regions.

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